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The first-in-class peptide binder to the SARS-CoV-2 spike protein

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28 **Abstract**

29 Coronavirus disease 19 (COVID-19) is an emerging global health crisis. With over 200,000
30 confirmed cases to date, this pandemic continues to expand, spurring research to discover
31 vaccines and therapies. SARS-CoV-2 is the novel coronavirus responsible for this disease. It
32 initiates entry into human cells by binding to angiotensin-converting enzyme 2 (ACE2) via the
33 receptor binding domain (RBD) of its spike protein (S). Disrupting the SARS-CoV-2-RBD binding
34 to ACE2 with designer drugs has the potential to inhibit the virus from entering human cells,
35 presenting a new modality for therapeutic intervention. Peptide-based binders are an attractive
36 solution to inhibit the RBD-ACE2 interaction by adequately covering the extended protein contact
37 interface. Using molecular dynamics simulations based on the recently solved ACE2 and SARS-
38 CoV-2-RBD co-crystal structure, we observed that the ACE2 peptidase domain (PD) α 1 helix is
39 important for binding SARS-CoV-2-RBD. Using automated fast-flow peptide synthesis, we
40 chemically synthesized a 23-mer peptide fragment of the ACE2 PD α 1 helix composed entirely of
41 proteinogenic amino acids. Chemical synthesis of this human derived sequence was complete in
42 1.5 hours and after work up and isolation >20 milligrams of pure material was obtained. Bio-layer
43 interferometry revealed that this peptide specifically associates with the SARS-CoV-2-RBD with
44 low nanomolar affinity. This peptide binder to SARS-CoV-2-RBD provides new avenues for
45 COVID-19 treatment and diagnostic modalities by blocking the SARS-CoV-2 spike protein
46 interaction with ACE2 and thus precluding virus entry into human cells.

47

48 **Key words**: SARS-CoV-2, peptide binder, protein-protein interaction inhibitor, coronavirus,
49 COVID-19, rapid response, peptide therapeutic, MD simulation, automated flow peptide synthesis

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52 **1. Introduction**

53 A novel coronavirus (SARS-CoV-2) from Wuhan, China, has caused 207,855 confirmed
54 cases and 8,648 deaths globally, according to the COVID-19 situation report from WHO on Mar
55 19, 2020 (<https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports/>),
56 and the number is continually growing. Similar to the SARS-CoV outbreak in 2002, SARS-CoV-2
57 causes severe respiratory problems. Coughing, fever, difficulties in breathing and/or shortage of
58 breath are the common symptoms. Aged patients with pre-existing medical conditions are at most
59 risk with a mortality rate ~1.5% or even higher in some regions. Moreover, human-to-human
60 transmission can occur rapidly by close contact. To slow this pandemic and treat infected patients,
61 rapid development of specific antiviral drugs is of the highest urgency.

62 The closely-related SARS-CoV coronavirus invades host cells by binding the angiotensin-
63 converting enzyme 2 (ACE2) receptor on human cell surface through its viral spike protein (S) [1-
64 4]. It was recently established that SARS-CoV-2 uses the same receptor for host cell entry [5, 6].
65 Recent crystallographic studies of the SARS-CoV-2-S receptor binding domain (RBD) and full-
66 length human ACE2 receptor revealed key amino acid residues at the contact interface between
67 the two proteins and provide valuable structural information that can be leveraged for the
68 development of disruptors specific for the SARS-CoV-2/ACE2 protein-protein interaction (PPI) [7,
69 8]. Small-molecule inhibitors are often less effective at disrupting extended protein binding
70 interfaces [9]. Peptides, on the other hand, offer a synthetically accessible solution to disrupt PPIs
71 by binding at interface regions containing multiple contact “hot spots” [10].

72 We hypothesize that disruption of the viral SARS-CoV-2-RBD-host ACE2 interaction with
73 peptide-based binders will prevent virus entry into human cells, offering a novel opportunity for
74 therapeutic intervention. Toward this aim, we launched a campaign to rapidly discover peptide
75 binders to SARS-CoV-2-RBD. Analyzing the RBD-ACE2 co-crystal structure, we found that
76 SARS-CoV-2-RBD/ACE2 interface spans a large elongated surface area, as is common for PPIs.

77 We leveraged molecular dynamic simulations and automated fast-flow peptide synthesis [11] to
78 prepare a 23-mer peptide binder (SBP1) to SARS-CoV-2-RBD, the sequence of which was
79 derived from the ACE2 α 1 helix. Using bio-layer interferometry, we determined that SBP1 binds
80 SARS-CoV-2-RBD with low nanomolar affinity (dissociation constant, $K_D = 47$ nM). SBP1 is a
81 first-in-class peptide binder to SARS-CoV-2-RBD, potentially inhibiting entry of the virus into
82 human cells. In addition, the human protein-derived sequence of SBP1 is fully proteinogenic, and
83 is not expected to be immunogenic. Taken together, computational simulations coupled with
84 automated flow peptide synthesis technology enable an accelerated discovery loop from design
85 to experimental validation, and rapidly delivered SBP1 as a promising pre-clinical drug lead.
86 Antiviral activity studies in mammalian cell cultures are in progress in order to evaluate efficacy
87 of the current and future optimized peptide binders.

88

89 **2. Results**

90

91 **Molecular dynamic simulations guide peptide binder design**

92 Using the Amber force field [12], a helical peptide sequence (spike-binding peptide 1,
93 SBP1) derived from the α 1 helix of ACE2 peptidase domain (ACE2-PD) in complex with SARS-
94 CoV-2-RBD was simulated under TIP3P explicit water conditions. Analyzing the simulation
95 trajectory after 200 ns, we found that SBP1 remains on the spike RBD protein surface in a stable
96 conformation (Fig. 2B) with overall residue fluctuations smaller than 0.8 nm compared with their
97 starting coordinates (Fig. 2A). Per-residue analysis along the 200 ns trajectory showed that the
98 middle residues of SBP1, a sequence we termed SBP2, have significantly reduced fluctuations
99 (Fig. 2C, 2D), indicating key interactions. The results of this MD simulation suggest that SBP1
100 and SBP2 peptides derived from the ACE-PD α 1 helix may alone potentially bind the SARS-CoV-
101 2 spike RBD protein with sufficient affinity to disrupt the associated PPI.

102 **Automated fast-flow peptide synthesis yielded >95% pure compound**

103 The two biotinylated peptides, SBP1 and SBP2, derived from the α 1 helix were prepared
104 by automated fast-flow peptide synthesis [11, 13] with a total synthesis time of 1.5 h for a total of
105 35 couplings. After cleavage from resin, global deprotection, and subsequent C18 solid-phase
106 extraction, the purity of the crude peptides was estimated to be >95% for both biotinylated SBP1
107 and SBP2 based on LC-MS TIC chromatograms (supplemental Fig. 1A and 1B). We assessed
108 this purity as acceptable for direct downstream biological characterizations.

109 **SBP1 peptide specifically binds SARS-CoV-2-RBD with low nanomolar affinity**

110 Bio-layer interferometry was employed to measure the affinity of synthesized peptides
111 (e.g., SBP1) to glycosylated SARS-CoV-2-RBD or the human protein menin as a negative control.
112 Biotinylated peptide was immobilized onto streptavidin (SA) biosensors. After fitting the
113 association and dissociation curves from serial dilutions of protein, the dissociation constant (K_D)
114 of SBP1 to the RBD was determined to be 47 nM with the average $K_{on} = 4.69 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$ and
115 $K_{off} = 2.2 \times 10^{-3} \text{ s}^{-1}$ (Fig. 2E). However, SBP2 (a truncate of SBP1) did not associate with the spike
116 RBD protein (Fig. 2F). SBP1 had no observable binding to the negative control human protein
117 menin (Fig. 2G). Together, these data indicate that SBP1 specifically binds SARS-CoV-2-RBD
118 with low nanomolar affinity.

119

120 **3. Discussion**

121 Recently published cryo-EM and co-crystal structures of the RBD of SARS-CoV-2 with
122 human ACE2 have identified this PPI as key step for the entry of SARS-CoV-2 to human cells.
123 Blocking this binding interface represents a highly promising therapeutic strategy, as it could
124 potentially hinder SARS-CoV-2 from entering cells and replicating.

125 Drugging PPIs is a longstanding challenge in traditional drug discovery and peptide-based
126 approaches might help to solve this problem. Small molecule compounds are unlikely to bind
127 large protein surfaces that do not have distinct binding pockets. Peptides, on the other hand,
128 display a larger surface area and chemical functionalities that can mimic and disrupt the native
129 PPI, as is the case for the clinically approved HIV peptide drug Fuzeon [14, 15].

130 The identification of a suitable starting point for drug discovery campaigns can be time-
131 intensive. During a pandemic such as this one, therapeutic interventions are urgently needed. To
132 rapidly identify potential peptide binders to the SARS-CoV-2 spike protein, we used molecular
133 dynamics (MD) simulation on peptides extracted from the human ACE2 sequence. The starting
134 point of the binding simulations was the cryo-electron microscopy model of SARS-CoV-2 spike
135 protein and several peptides derived from the SARS-CoV-2-spike binding domain of human ACE2
136 protein. Our MD simulation (200 ns trajectory) indicated that the SBP1 peptide, corresponding to
137 the *N*-terminal ACE2 α 1 helix, stably bound to SARS-CoV-2-RBD. The overall peptide fluctuations
138 were smaller than 0.8 nm from starting coordinates (Fig. 2A). These results indicated the potential
139 of identifying a SARS-CoV-2-RBD-binding peptide derived from the human ACE2 α 1 helix.

140 A 23-mer peptide sequence (SBP1) was synthesized by automated flow peptide synthesis.
141 The 23 residues selected from the ACE2 α 1 helix sequence (IEEQAKTFLDKFNHEAEDLFYQS)
142 showed low fluctuations along the MD simulation trajectory and several important interactions
143 with the spike protein were observed consistently with multiple lines of published data [8, 16]. We
144 decided to use this peptide (SBP1) as an experimental starting point for the development of a
145 SARS-CoV-2 spike protein binder. Our rapid automated flow peptide synthesizer [11, 13] enabled
146 the synthesis of tens of milligrams of SBP1 peptide within minutes. The crude purity was
147 determined to be >95% and therefore sufficient for binding validation by bio-layer interferometry
148 (BLI).

149 The interaction between SBP1 and the RBD of SARS-CoV-2 spike protein was validated
150 by bio-layer interferometry. The K_D , derived from protein association and dissociation kinetics,
151 was found to be 47 nM after averaging the fitting values at different protein concentrations (Fig.
152 2E). This binding affinity is comparable with that of full length ACE2 binding to SARS-CoV-2-RBD
153 (14.7 nM) [7]. Excess of SBP1 therefore, could potentially cover spike proteins on SARS-CoV-2
154 surface and outcompete the binding for ACE2. Further optimization of the sequence length and
155 amino acid composition are in progress, in order produce binders with higher PPI inhibitory activity.

156 SBP1 is a “fully human,” endogenous peptide and therefore likely to be well tolerated by
157 the human immune system. The amino acid sequence of SBP1 is entirely derived from human
158 ACE2 and should be recognized as endogenous by the human immune system. This feature
159 could be highly beneficial in later stages of pre-clinical development. Challenges associated with
160 peptide drugs, such as proteolytic degradation and rapid renal elimination, will be addressed in
161 the near future by chemical modifications of the sequence.

162 In conclusion, a peptide sequence derived from human ACE2 was found to bind the
163 SARS-CoV-2 spike protein RBD with low nanomolar affinity. We believe disruption of the SARS-
164 CoV-2-RBD/ACE2 binding interface with high-affinity peptides represents a promising strategy for
165 preventing virus entry in human cells and paves the way for new COVID-19 treatment and
166 diagnostic modalities. Upon request, we will make available our peptide binder and all upcoming
167 optimized variants to research facilities testing and developing potential COVID-19 treatments.
168 Rapid discovery, synthesis, and testing of potential drug leads should help the global scientific
169 and healthcare communities efficiently address the ongoing crisis.

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173 **4. Experimental Materials and Methods**

174 **GPU-accelerated molecular dynamic simulation**

175 The co-crystal structure of SARS-CoV-2-RBD with ACE2-B⁰AT1 (PDB: 6M17) was chosen as the
176 initial structure, which was explicitly solvated in an 87 Å³ box, to perform a 200 ns molecular
177 dynamical (MD) simulation using NAMD on MIT's supercomputing clusters (GPU node). Amber
178 force field was used to model the protein and peptide. The MD simulation system was equilibrated
179 at 300 K for 2 ns. Periodic boundary conditions were used and long-range electrostatic
180 interactions were calculated with particle mesh Ewald method, with non-bonded cutoff set to 12.0
181 Å. SHAKE algorithm was used to constrain bonds involving hydrogen atoms. Time step is 2 fs
182 and the trajectories were recorded every 10 ps. After simulation production runs, trajectory files
183 were loaded into the VMD software for further analysis.

184 **Automated fast-flow peptide synthesis**

185 SBP1 and SBP2 sequences were synthesized at 90 °C on Rink Amide-ChemMatrix resin with
186 HATU activation using a fully automatic flow-based peptide synthesizer. Amide bond formation
187 was performed in 8 seconds, and Fmoc groups were removed in 8 seconds with 40% (v/v)
188 piperidine in DMF. Overall cycle times were about 120 seconds. After completion of fast-flow
189 synthesis, the resins were washed with DMF (3 x) and then incubated with HATU-activated biotin-
190 PEG₄-propionic acid (CAS# 721431-18-1) at room temperature for 1.0 h for biotinylation on the
191 peptide *N*-terminus.

192 **Peptide cleavage and deprotection**

193 After peptide synthesis, the peptidyl resin was rinsed with dichloromethane briefly and then dried
194 in a vacuum chamber overnight. Next day, approximately 5 mL of cleavage solution (94% TFA,
195 1% TIPS, 2.5% EDT, 2.5% water) was added into the syringe containing the resin. The syringe
196 was kept at room temperature for 2 h before injecting the cleavage solution into a 50 mL conical

197 tube. Dry-ice cold diethyl ether (~50 mL) was added to the cleavage mixture and the precipitate
198 was collected by centrifugation and triturated twice with cold diethyl ether (50 mL). The
199 supernatant was discarded. Residual ether was allowed to evaporate and the peptide was
200 dissolved in water with 0.1% TFA for solid-phase extraction.

201 **Solid-phase extraction (SPE)**

202 After peptide cleavage, peptide precipitates were dissolved in water with 0.1% TFA. Agilent Mega
203 BE C18 column (Part No: 12256130) was conditioned with 5 mL of 100% acetonitrile with 0.1%
204 TFA, and then equilibrated with 15 mL of water with 0.1% TFA. Peptides were loaded onto the
205 column for binding, followed by washing with 15 mL of water with 0.1% TFA, and finally, eluted
206 with 5 mL of 30/70 water/acetonitrile (v/v) with 0.1% TFA.

207 **Liquid chromatography-mass spectrometry (LC-MS)**

208 Peptides were dissolved in water with 0.1% TFA followed by LC-MS analysis on an Agilent 6550
209 ESI-Q-TOF instrument using an Agilent Jupiter C4 reverse-phase column (2.1 mm × 150 mm, 5
210 µm particle size). Mobile phases were 0.1% formic acid in water (solvent A) and 0.1% formic acid
211 in acetonitrile (solvent B). Linear gradients of 1 to 61% solvent B over 15 minutes (flow rate: 0.5
212 mL/min) were used to acquire LC-MS chromatograms.

213 **Kinetic binding assay using bio-layer interferometry (BLI)**

214 A ForteBio Octet® RED96 Bio-Layer Interferometry system (Octet RED96, ForteBio, CA) was
215 used to characterize the *in vitro* peptide-protein binding at 30 °C and 1000 rpm. Briefly,
216 streptavidin (SA) tips were dipped in 200 µL of biotinylated peptide solution (2.5 µM in 1x kinetic
217 buffer: 1x PBS with 0.1% BSA and 0.05% tween) for the loading step. The tips loaded with peptide
218 were then sampled with SARS-CoV-2-RBD or menin protein at various concentrations in 1x
219 kinetic buffer to obtain the association curve. Peptide only was used as reference for background
220 subtraction. After association, the tips were dipped back into 1x kinetic buffer to obtain the

221 dissociation curve. The association and dissociation curves were fitted with ForteBio Biosystems
222 using four experimental conditions ($n = 4$, global fitting algorithm, binding model 1:1) to obtain the
223 dissociation constant K_D .

224

225 **Acknowledgements**

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227 resources to run MD simulations. This research was supported by MIT start-up and seed funds.
228 S.P. is supported by a postdoctoral fellowship from Deutsche Forschungsgemeinschaft (award
229 PO 2413/1-1). MIT has filed a provisional patent application related to this work.

230 **Competing interests**

231 B.L.P. is a founder of Resolute Bio and Amide Technologies.

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233

234 **Figure captions:**

235 **Figure 1. MD-guided target selection for rapid flow synthesis of a SARS-CoV-2-RBD-**
236 **binding peptide.** Fragments of ACE2-PD domain are docked against SARS-CoV-2 receptor-
237 binding domain (PDB: 6M17). Low RMSD peptides are rapidly synthesized by fully automated
238 flow peptide synthesis, and binding to glycosylated SARS-CoV-2-RBD is determined by BLI.

239

240 **Figure 2. Human ACE2-PD domain α -helix 1-derived SBP1 binds SARS-CoV-2-RBD.** (A)
241 RMSD for SBP1 docked to SARS-CoV-2-RBD during 200 ns MD simulation. (B) Binding interface
242 between SARS-CoV-2-RBD and SBP1 after 200 ns simulation. Individual RMSD (C) and average
243 RMSD (D) values for SBP1 residues over the course of the 200 ns simulation. Arrows indicate
244 residues contributing key hydrogen bonding interactions (determined using UCSF Chimera,
245 Version 1.12). Individual residues with RMSD below 5 Å arbitrarily colored green. Binding affinity
246 of SBP1 and SBP2 to glycosylated SARS-CoV-2-RBD (E, F), and affinity of SBP1 to negative
247 control human protein menin (G), as determined by bio-layer interferometry.

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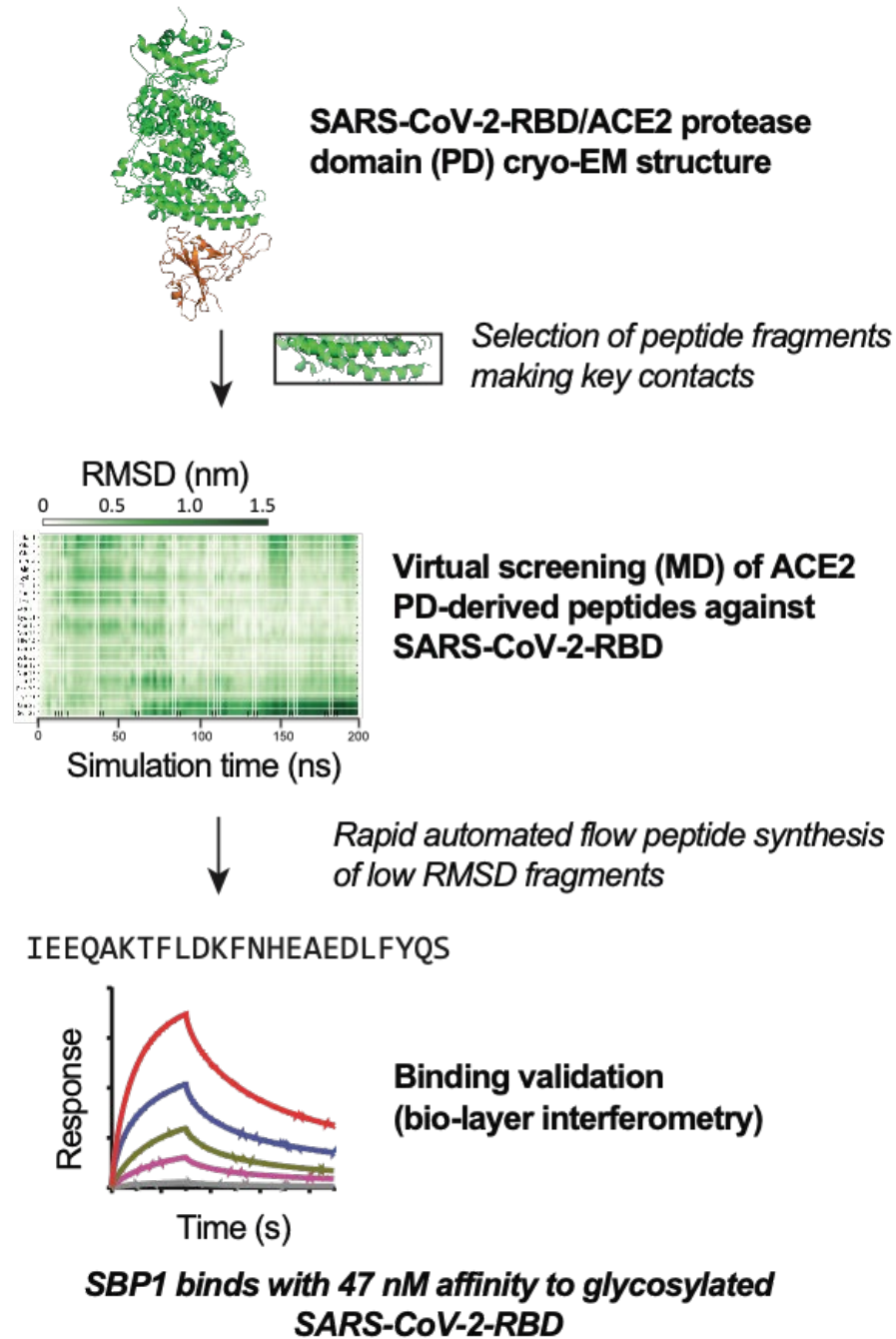
249 **Supplemental Figure 1. ACE2-derived peptides were prepared by solid-phase peptide**
250 **synthesis.** Total ion current chromatograms (TIC) of purified biotinylated SBP1 peptide (A),
251 purified biotinylated SBP2 peptide (B), crude SBP1 peptide (C), and crude SBP2 peptide (D).

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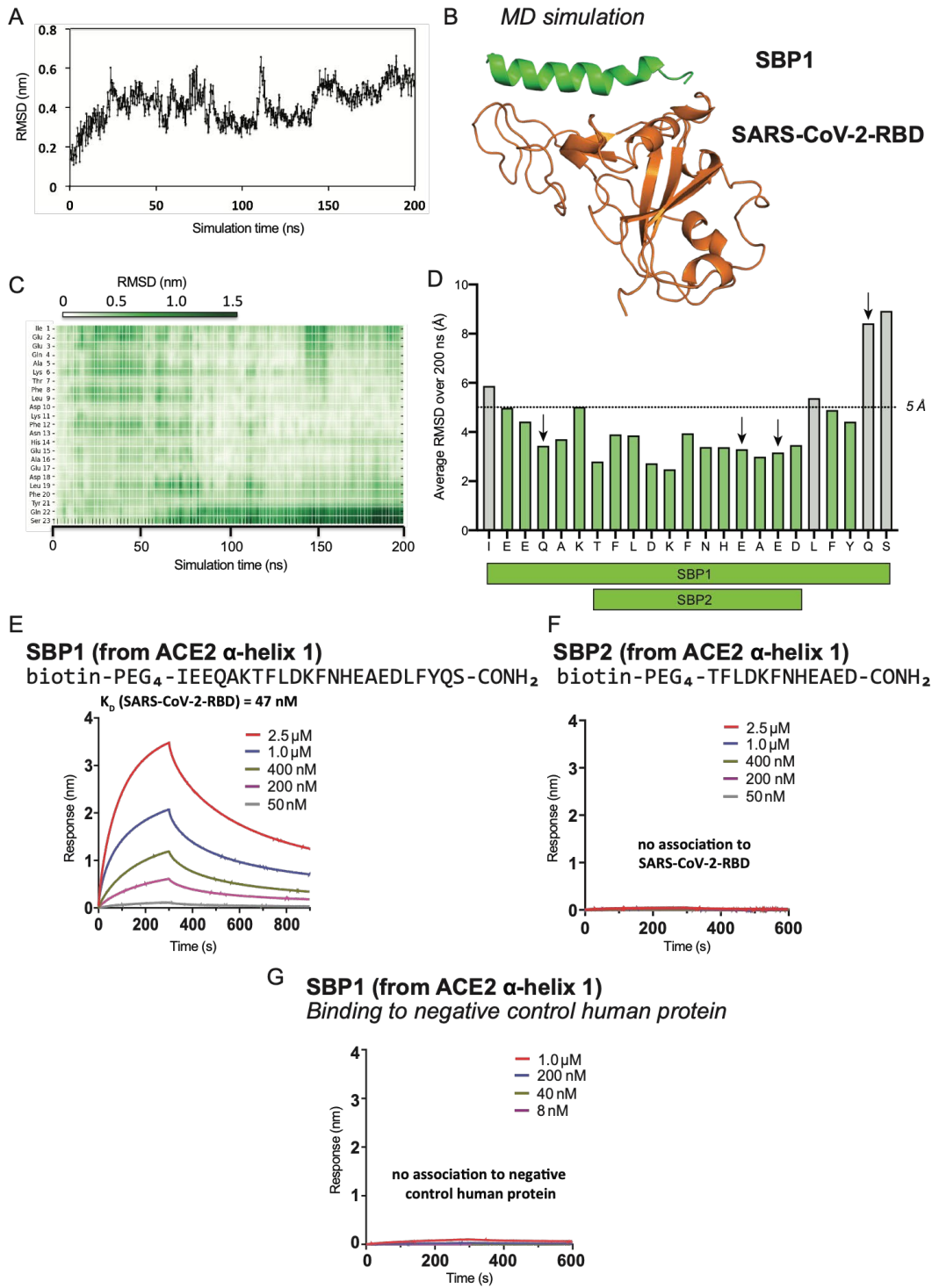
254 **Figures:**

255 **Figure 1**



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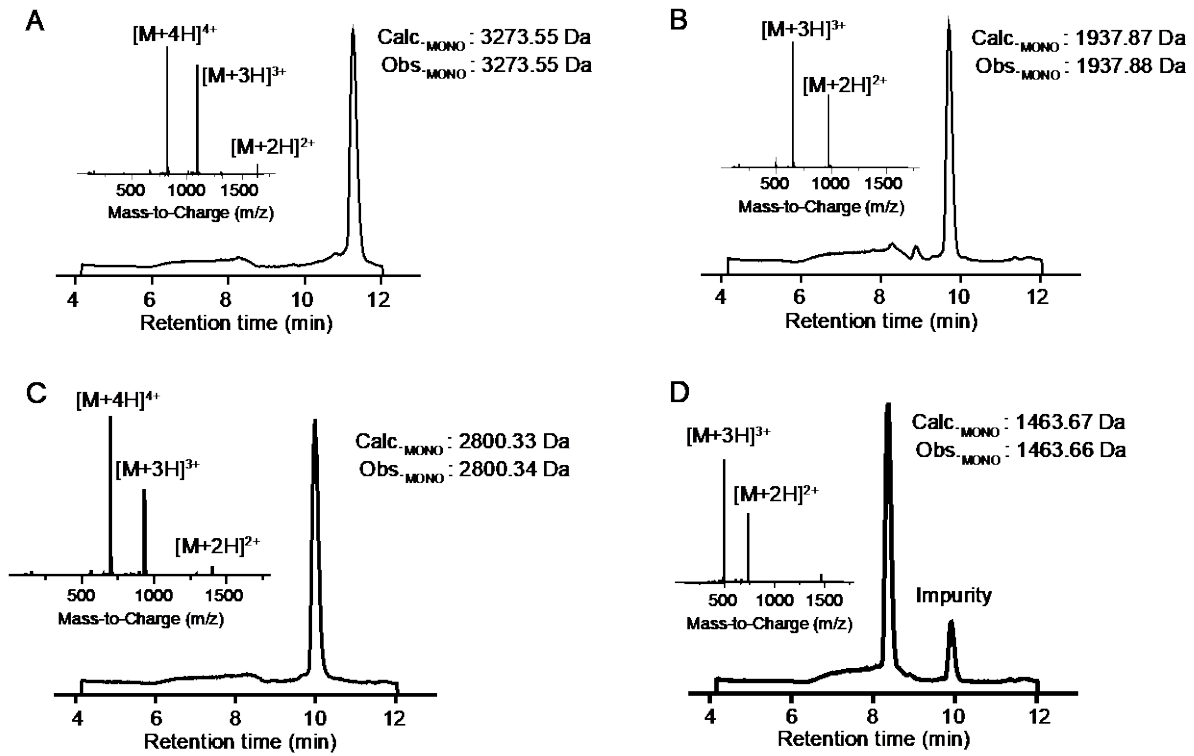
257 **Figure 2**



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260 **Supplemental Figure 1**



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